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NASA Grant NAG-2-100

NEURAL CONTROL OF MUSCLE ANDROGEN RECEPTORS

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Abbreviations used: R1881, methyltrienolone; E_2, estradiol-17β benzoate; TP, testosterone propionate; OVX, ovariectomy; GDX, orchiectomy; TA, tibialis anterior muscle; EDL, extensor digitorum longus muscle

Abstract

The number of cytosolic androgen receptors in rat skeletal muscle increases following denervation and disuse (J. Neurochem. 43:1479-1483, 1984). This increase was postulated to represent altered intracellular distribution and consequent diminished sensitivity of skeletal muscle to androgens. To test this hypothesis, we measured total (homogenate) androgen receptor levels after denervation. Total (homogenate) androgen receptor binding did not change in response to denervation of leg muscles from adult male rats. An increase in cytosolic receptor number with no increase in total (homogenate) receptor levels supports the hypothesis of altered intracellular distribution of androgen receptors in denervated muscle. Cytosolic androgen receptor binding in muscle from male rats increased by 40% after denervation, while in females, the increase was 17%. These increases could not be altered by endocrine manipulations of males or females.

Short Title: Muscle Androgen Receptors

Key Words: muscle, androgen receptors, denervation

Introduction

The actions of androgens on striated muscle appear to be mediated via interaction of these hormones with intracellular receptors (Max, 1981; Max et al., 1981; Max, 1983; Krieg, 1980). The concentration of such receptors is, therefore, likely to be an important determinant of the androgen sensitivity of muscle (Baxter and Funder, 1979). Androgen receptors in muscle have been shown to be subject to endocrine (Rance and Max, 1984) and neural (Bernard et al., 1984) control. Regarding neural control, we (Bernard et al., 1984) showed that denervation and disuse caused an increase in cytosolic androgen receptor binding in rat hind-limb and levator ani muscles. We hypothesized that increased muscle cytosolic androgen receptor binding following denervation reflected altered intracellular distribution of androgen receptors, rather than increased receptor synthesis. We now report evidence to support this hypothesis. Further, we describe the response to denervation of androgen receptors in muscle from male and female rats.

Materials and Methods

Animals - Rats of both sexes of the CD strain (Charles River Breeding Labs, Wilmington, MA) were used. They were housed in a 12 h lights on:12 h lights off cycle, and they were fed Purina Rodent Laboratory Chow (#5001) and water ad libitum. Adult males and females weighed 150-200 g; immature (< 3 weeks old) rats weighed 40-50 g. Denervation was achieved by section of the sciatic nerve in the mid-thigh region while the rats were under ether anesthesia. Following neurotomy, the underlying muscles

were apposed with sutures, and the skin wound was closed with wound clips. Ovariectomy (OVX) or orchiectomy (GDX) were performed under ether anesthesia, via dorsal and abdominal routes, respectively.

Androgen Receptor Binding - This was assessed using [^{3}H] methyltrienolone (17-hydroxy-17α-[^{3}H]methyl-4,9,11-estratrien-3-one; R1881; specific activity 86 mCi/mmole; New England Nuclear, Waltham, MA). We assessed androgen receptor binding using a novel filtration assay employing hydroxylapatite (Bio-Gel HTP, BioPad, Richmond, CA), to separate bound and unbound ligand (Liao et al., 1984). This procedure resulted in higher values for specific binding to the cytosolic androgen receptor in muscle than we have reported (Bernard et al., 1984). Cytosol receptor binding was done at a single, saturating concentration (15 nM) using the incubation conditions described (Pance and Max, 1984). We also measured total muscle (i.e., homogenate) androgen receptor binding by the following procedure. Minced muscle was homogenized in a polytron homogenizer in ice-cold buffer (5 ml/g muscle) comprising 25 mM Na_2HPO_4, 1.5 mM EDTA, 10% (v/v) glycerol, 2 mM dithiothreitol, 10 mM sodium molybdate, and 1 mM phenylmethylsulfonyl fluoride, pH 7.4. The homogenate was filtered through a 40 mesh Nitex screen to remove connective tissue particles. Homogenate binding was performed both with single-point assays (15 nM) and by Scatchard analysis (Scatchard, 1949). It was necessary to determine optimal conditions for exchange in the homogenate assays, because this has not been done heretofore with muscle. Fig. 1 shows that [^{3}H] methyltrienolone

[illegible] at 0-4°. However, [illegible] at 24 h at 0-4° when occupied muscle androgen receptor (prepared from castrate muscle homogenate incubated with 5 nM [^{3}H] 5α-dihydrotestosterone for 4 h) was incubated with 15 nM methyltrienolone and diluted four-fold.

Protein was determined according to Lowry et al. (1952).

Steroid Hormone Administration to Rats - This was accomplished by subcutaneous implantation of Silastic capsules containing estradiol-17β benzoate dissolved in sesame oil, or solid testosterone propionate, as described (Bance and Max, 1984).

Chemicals - Unless otherwise stated, all chemicals were from Sigma (St. Louis, MO).

Data analysis - Statistical analysis was performed with a t-test. Scatchard plot data were computed with the program of McPhersen (1983), using an IBM Personal Computer.

Results

Total (homogenate) androgen receptor binding in denervated and control muscle:

Nerve section causes a significant (40-50%) increase in cytosol androgen receptor binding (Bernard et al., 1984). Because it was not blocked by cycloheximide, the increase did not appear to reflect enhanced receptor synthesis; rather, altered intracellular distribution was proposed. Fig. 2 shows that Scatchard graphs of total homogenate androgen receptor binding of denervated and control rat tibialis anterior and extensor digitorum longus (EDL/TA) muscles did not differ. This conclusion

is supported by the single-point data of Table I. On the other hand, cytosol receptor binding was increased following denervation (Table II, Fig. 3) in agreement with our earlier result (Berrard et al., 1984).

We then compared the effect of denervation of EDL/TA muscles on cytosolic androgen receptor binding in male and female rats. The basal level of cytosol receptor binding in muscle from female rats is greater than that from males (Table II), in agreement with Dahlberg et al. (1981). As expected, denervation caused a significant (40%) increase in cytosolic androgen receptor binding in muscle from male rats (Table II). The denervation-induced increase in cytosolic androgen receptor binding from intact adult female rats was also significant, but amounted to only about 17% (Table II).

It was not possible to alter appreciably the male or female responses to denervation by endocrine manipulations, either of immature or mature males and females. The data for males are summarized in Fig. 2. They are presented as % control because differences in the basal level of binding among male, female, adult, immature, and castrated rats (Dahlberg et al., 1981) make comparisons in absolute terms difficult. Thus, as noted above, denervation of adult males increased cytosolic androgen receptor binding by about 40%. Denervation of EDL/TA from immature males caused a 30% increase in cytosolic androgen receptor binding. Treatment of immature male rats with estradiol-17β for 4 weeks prior to denervation also resulted in a 30% increase in [^{3}H] R1881 binding. Castration at < 3 weeks of age resulted in androgen

receptor binding that was 52% greater than control following denervation 4 weeks later.

Similarly, endocrine manipulations of female rats did not alter the response of muscle androgen receptors to denervation (Fig. 3). Thus, denervation of EDL/TA in normal adults, ovariectomized adult females, or immature females who received TP implants for 3 weeks prior to denervation, caused a 17% increase in cytosolic androgen receptor binding 24 h following denervation in every case.

Discussion

The data described above add strong support to our contention that denervation of skeletal muscle causes an increase in cytosolic androgen receptor binding as a result of altered intracellular distribution (Bernard et al., 1984). This argument is based on the following data: cytosolic androgen receptor binding increased by about 40-50% following nerve section (Bernard et al., 1984; Table II; Fig. 3). This increase was not blocked by cycloheximide (Bernard et al., 1984), suggesting that de novo receptor synthesis is not involved. Further, injection of TP 1 h prior to killing rats whose hind-limb had been denervated 23 h previously caused some loss of cytosol receptor from denervated muscle, but the loss was smaller than the decrease in cytosolic receptor binding in innervated muscle following TP injection (Bernard et al., 1984). This type of experiment may be interpreted as reflecting increased nuclear binding with concomitant loss of receptor from the cytosol (e.g., Isomaa et al., 1982). Further, the present data (Fig. 2, Table I), show

that total muscle (homogenate) androgen receptor binding is not increased by denervation. Recent data suggest that unoccupied receptors may exist in the nucleus, in vivo, and that the presence of receptor in the cytosol is a consequence of homogenization (Jordan et al., 1985). According to this view, the phenomenon described herein might reflect altered affinity of the receptor for nuclear components such that more receptor is released into the cytosol during homogenization. A change in nuclear affinity apparently accounts for a refractory period for progesterone receptor binding during development of the chick oviduct (Boyd-Leinen, 1984). Furthermore, Sibley and Tomkins (1974) described mutant cells that were resistant to the actions of glucocorticoids. Among these mutants were cells that could not translocate or bind hormone receptors in the nucleus.

A change in intracellular distribution of the androgen receptor might reflect altered androgen sensitivity of denervated muscle. Indeed, diminished androgen sensitivity has been reported for denervated rat levator ani muscle (Kare et al., 1985; Buresova et al., 1972; Pagni et al., 1973) and for avian syringeal muscle (Bleisch et al., 1973). Buresova et al. (1973), however, reported that the loss of androgen sensitivity of levator ani muscle following denervation was not noted in immature (< 3 weeks old) rats, but only in adults. In our study, muscle from immature male rats displayed the same increase in muscle androgen receptor binding following denervation as adults (Fig. 2).

Diminished androgen sensitivity might explain the apparent lack of beneficial effects of androgens in patients with

motoneuron diseases such as amyotrophic lateral sclerosis (Weiner et al., 1980). The incidence of motoneuron disease is higher in males than females (Weiner, 1980 ; Hausmanova-Petrusewicz et al., 1982). It is possible that the small sex difference in the response of muscle androgen receptors to denervation (Table II, Figs. 2 and 3) might be related to this clinical phenomenon. We were unable to alter the male/female difference in response of muscle androgen receptors to denervation by hormone manipulations from 3 weeks of age to adult. Perhaps perinatal estrogen (Döhler and Wuttke, 1975; Döhler et al., 1984) imparts the male/female difference noted above.

Acknowledgement

We thank Dr. J. T. Tildon for helpful comments, Ms. M. Wennes for technical assistance, and Ms. B. Pasko for preparation of the typescript. The work was supported by grants from NASA (NAG 2-100) NIH (NS-15760 and NS-20022) and by the Veterans Administration Research Service.

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Figure Legends

Figure 1 - Exchange conditions for total (homogenate) androgen receptor binding by rat skeletal muscle. Experimental procedures are described in the text.

Figure 2 - Scatchard analysis of [^{3}H] methyltrienolone specific binding by homogenates of control and denervated (24 h) tibialis anterior and extensor digitorum longus muscles from adult male rats. There was no significant difference in K_D or Bmax. Experimental procedures are described in the text.

Figure 3 - Effect of denervation (24 h) on cytosolic androgen receptor binding of extensor digitorum longus and tibialis anterior muscles from male rats. Data are presented as average % of the value from contralateral control muscles ± SD. *Significantly different from control, $p < 0.05$. 3 wk., < 50 g body wt.; 3 wk. $^{+}E_2$ → adult, rats received Silastic capsules containing estradiol-17β at 3 weeks of age - 4 weeks later, tibialis anterior and extensor digitorum longus muscles were denervated (24 h); GDX 3 wk → adult, rats were orchiectomized at 3 weeks of age - 4 weeks later, extensor digitorum longus and anterior tibialis muscles were denervated (24 h). Experimental procedures are described in the text.

Figure 4 - Effect of denervation (24 h) on cytosolic androgen receptor binding of extensor digitorum longus and tibialis anterior muscles from female rats. Data are presented as average % of the value for the contralateral control muscle ± SD. *Significantly different from control, $p < 0.05$. Adult + OVX, adult rats were ovariectomized 2 weeks prior to denervation; 3

weeks + TP → adult, rats were given Silastic capsules containing testosterone propionate at 3 weeks of age. Three weeks later, tibialis anterior and extensor digitorum longus muscles were denervated (24 h). Experimental details are provided in the text.

Table I

Effect of Denervation (24 h) on Homogenate Androgen Receptor Binding in Skeletal Muscle

	R1881 Specific Binding (fmols/g muscle)
control	171 ± 15 (6)
24 h denervation	178 ± 28 (6)

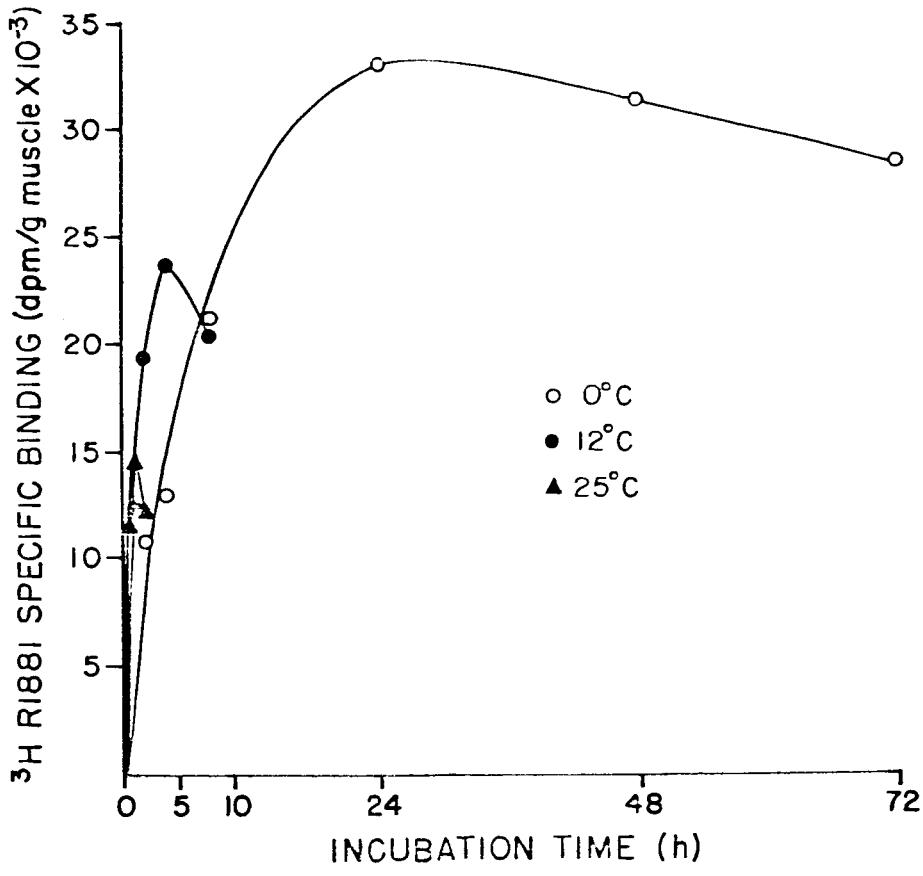
Experimental procedures are described in the text.

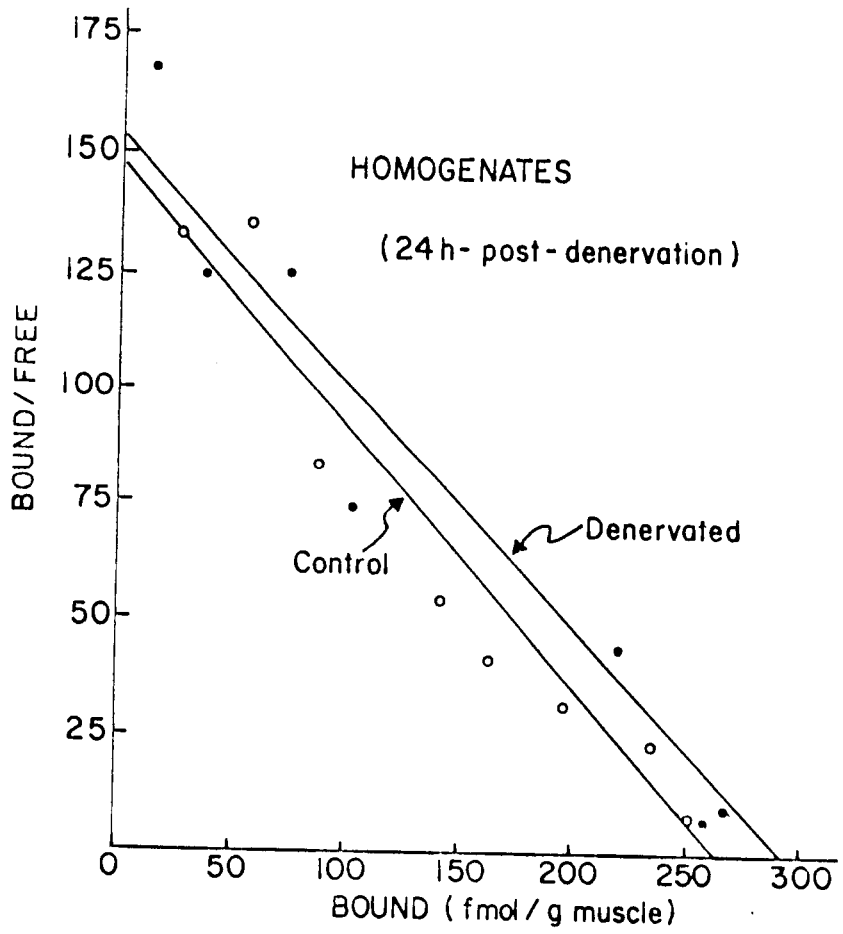
Table II

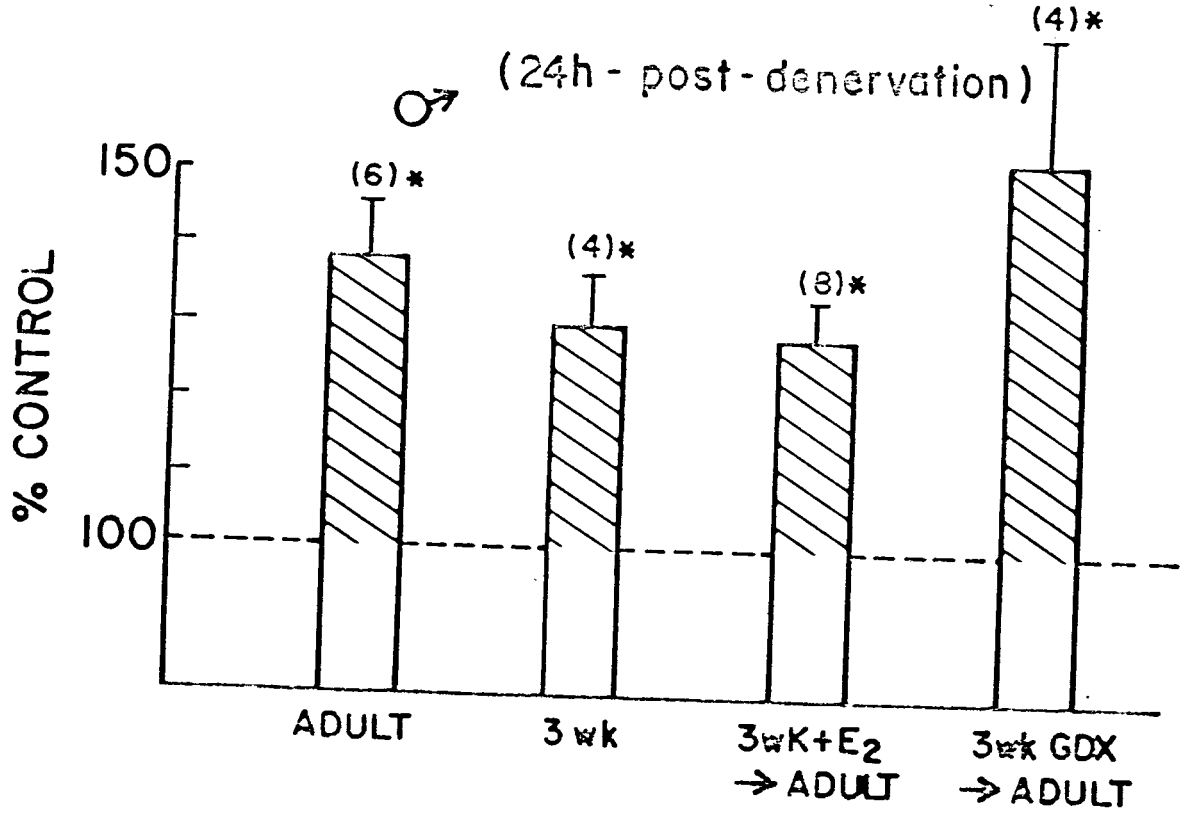
Effect of Denervation (24 h) on Cytosolic Androgen Receptor in Skeletal Muscle from Male and Female Rats

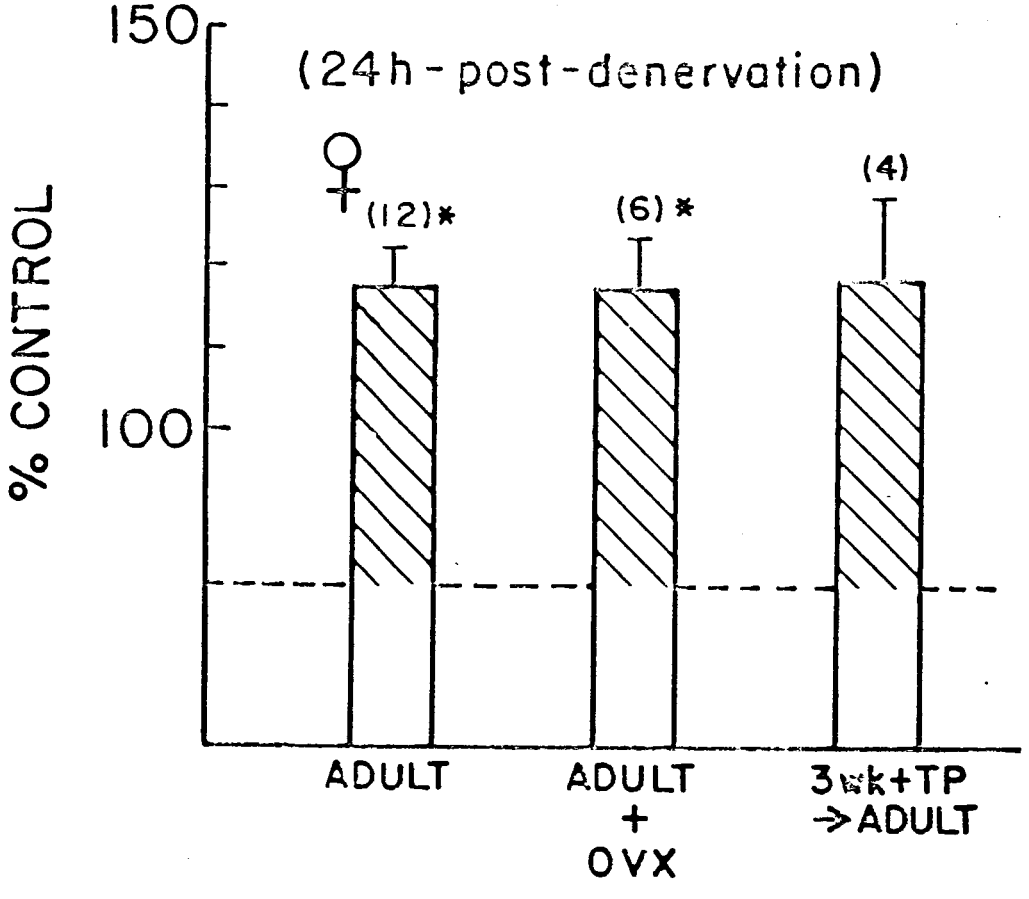
	R1881 Specific Binding (fmol/g muscle)		R1881 Specific Binding (fmol/mg protein)	
	Control	Denervated	Control	Denervated
Male	90 ± 6 (6)	126 ± 25 (6)**	2.03 ± 0.12 (6)	2.78 ± 0.52** (6)
Female	132 ± 15 (12)	157 ± 28 (10)*	2.69 ± 0.36 (12)	4.32 ± 0.68* (10)

Experimental procedures are described in the text. Significantly different from control, *$p < .01$; **$p < .005$.


^{3}H R1881 SPECIFIC BINDING (dpm/g muscle X 10^{-3})
35
30
25
20
15
10
5
0
5
10
24
48
72
INCUBATION TIME (h)
0°C
12°C
25°C


HOMOGENATES
(24 h- post-denervation)
BOUND/FREE
175
150
125
100
75
50
25
0
50
100
150
200
250
300
BOUND (fmol/g muscle)
Denervated
Control


(24h - post - denervation)
♂
150
100
% CONTROL
(6)*
(4)*
(8)*
(4)*
ADULT
3 wk
3wK+E2
→ ADULT
3wk GDX
→ ADULT


150
100
% CONTROL
(24h-post-denervation)
♀
(12)*
(6)*
(4)
ADULT
ADULT + OVX
3wk+TP →ADULT